

## EVIDENCE OF AN ASSEMBLY PHEROMONE IN THE BLACK-LEGGED DEER TICK, *Ixodes scapularis*

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(Received April 26, 2001; accepted August 31, 2001)

**Abstract**—The responses of *Ixodes scapularis* (Acari: Ixodidae) nymphs and adults to extracts of cast larval skins were tested in a Petri dish bioassay. Assembly was elicited in nymphs and adults in the presence of skins, exudate from ticks, and filter paper exposed to ticks compared to untreated controls. Assembly was noted by 1 hr after exposure with little change between 1 and 24 hr. The assembly response increased in the presence of an increased number of skins. Similar assembly was elicited in nymphs and adults in the presence of cast larval skins and a saline (0.95% NaCl) skin extract. Methanol and hexane extracts were not attractive. When chemical standards were tested against nymphs, they responded to guanine, uric acid, hypoxanthine, xanthine, inosine, and hematin. Adults were tested against guanine, inosine, and xanthine, and all elicited significant assembly. Responses of nymphs increased significantly with increase in dose of uric acid and guanine. Responses of nymphs to a mixture of guanine, xanthine, and adenine (25:1:1 ratio) were similar to responses to cast skins. This study provides the first evidence of an assembly pheromone in *I. scapularis*.

**Key Words**—Assembly pheromone, tick, *Ixodes scapularis*, purines, guanine.

### INTRODUCTION

Relatively little is known about the pheromonal regulation of behavior of the black-legged tick, *Ixodes scapularis* (Acari: Ixodidae) despite its public health importance as the primary vector of Lyme disease, human granulocytic ehrlichiosis, and human babesiosis in the United States (Spielman et al., 1985; Walker and

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Dumler, 1996; Childs et al., 1998). Reports of pheromones in other *Ixodes* species are also limited. Graf (1975) reported that a water-soluble pheromone produced by female *I. ricinus* strongly attracted males and, to a lesser degree, females. This pheromone appeared to facilitate mate finding and mating through an assembly response. Evidence of a water-soluble pheromone inducing assembly has also been documented for *Ixodes holocyclus* (Treverrow et al., 1977), *Ixodes persulcatus* (Uspensky and Emelyanova, 1980) and *I. ricinus* (Hájková and Leahy, 1982), however, these pheromones are yet to be characterized or identified.

Assembly pheromones typically induce formation of off-host clusters of various stages and sexes of ticks through arrestment of movement upon contact. These assemblies are best described in soft ticks and tend to occur in caves, under ledges, and in cracks and crevices in proximity to nests of the hosts. These assemblies are thought to enhance mating and host-finding success (Sonenshine et al., 1982). Pheromone-induced assembly was first described from *Argas* ticks by Leahy et al. (1973). Subsequently, this behavior has been reported from at least 14 species of soft ticks as well as from several hard ticks including *Hyalomma dromedarii* (Leahy et al., 1981), *Rhipicephalus appendiculatus*, *Amblyomma cohaerans* (Otieno et al., 1985), *Rhipicephalus evertsi* (Gothe and Neitz, 1985), and the above mentioned species of *Ixodes*. Assembly pheromones are present in the excreta and the aqueous and saline washings of ticks (Leahy et al., 1973; Gothe and Kraiss, 1982). Best described from several species of *Argas*, assembly pheromones appear to consist of guanine and other purines such as xanthine and hypoxanthine that are present in extracts of nitrogenous excreta of these ticks (Neitz and Gothe, 1984; Otieno et al., 1985; Gothe, 1987; Dusbábek et al., 1991a,b). The existence of similar pheromones has not been previously reported from *Ixodes scapularis* and the objective of this study was to determine if assembly pheromones are present in *Ixodes scapularis*.

#### METHODS AND MATERIALS

**Ticks.** Ticks were obtained from a laboratory colony maintained at the University of Florida. Adult ticks were fed on New Zealand white rabbits and immatures fed on rats. Ticks were held in screen-topped vials at  $24 \pm 1^\circ\text{C}$  and 93–96% relative humidity under a 16L:8D photoperiod.

**Bioassay Procedure.** Responses of ticks were evaluated in Petri dish bioassays modified from Leahy et al. (1973). Disposable plastic Petri dishes (100 mm diam.) were placed over similar-sized paper templates divided into six equal-sized sectors. Sectors of the templates were labeled from 1 to 6. Teflon discs of 1 cm diam. (Thomas Scientific, Swedesboro, New Jersey) were treated with treatment solutions or solvent (control) and placed in the center of sector 1. None of the other sectors in the Petri dish received a treatment or control disc. Treatments or

controls were placed on Teflon discs and the solvent allowed to dry before being placed in the Petri dish. After the disc was added to the dish, 10 ticks were added in the center of the dish and the dish placed in a high-humidity (>93% relative humidity) chamber. The location of ticks by sector was noted at 1, 2, and 24 hr after placement to evaluate the cumulative effect of the assembly. Initial bioassays determined that there was no location bias under these experimental conditions and that there was no significant difference in numbers of ticks present in any sector (data not presented).

**Bioassays.** Initial bioassays were conducted using cast skins collected from blood-fed larvae, filter paper exposed to blood-fed larvae, and the exudate from blood-fed larvae. Skins shed by emerged nymphs were collected from tick rearing vials within a week after being shed. Skins were collected using fine-tipped forceps or a paintbrush, and only whole skins were used for extracts. Filter paper strips (1 × 4 cm) were placed in tick rearing vials along with 10 freshly blood-fed larvae. After 30 days of exposure, filter strips were removed from the vials and evaluated in bioassays. Exudate from the sides of glass tick rearing vials was removed by scraping with forceps and collected for use in bioassays. Bioassays were conducted immediately after collection of skins, filter paper, or exudate. Bioassays were conducted by placing cast skins (10 or 25), folded contaminated filter paper strips, or exudate (0.5 mg) on a Teflon disc in the center of sector 1. Controls consisted of an untreated Teflon disc or clean filter paper.

Extracts of cast skins were made using solvents covering a range of polarities and bioassay responses compared to those using cast skins. Cast skins were placed in 500  $\mu$ l of 0.95% NaCl, vortexed three times for 20 sec, allowed to sit for 10 min, and then all liquid was transferred to a separate vial. Skins were washed twice times with 100  $\mu$ l of 0.95% NaCl, and washes were added to the original extract and reduced under N<sub>2</sub> to a concentration of 1 skin equivalent/ $\mu$ l. Similarly, skins were washed with methanol (HPLC grade) and the extract reduced to 1 skin equivalent/ $\mu$ l. An extract with hexane (HPLC grade) was also prepared following the protocol for the above extracts. Bioassays were conducted comparing responses of *I. scapularis* females, males, and nymphs to cast larval skins, saline, methanol, and hexane extracts and untreated controls. Treatments were tested at 10 and 20 skins or skin equivalents. All bioassays were replicated 15 times.

Responses of *I. scapularis* were tested to a series of compounds previously reported as potential components of an assembly pheromone in soft ticks (Dusbábek et al., 1991a,b). Compounds tested included guanine, uric acid, hypoxanthine, xanthine, inosine, adenine, and hematin (Sigma, St. Louis, Missouri). Compounds were diluted in 0.95% NaCl and tested at 0.5 mg, which was a dose eliciting response in the study by Dusbábek et al. (1991a). Controls consisted of 1  $\mu$ l of 0.95% NaCl. Prior to bioassays, Teflon discs were treated with 0.5 mg of each compound,

discs were allowed to air dry, and then bioassays were conducted. Bioassays were replicated 10 times for nymphs and 15 times for adults.

Compounds eliciting significant responses were tested over a range of doses to examine dose-response relationships. Responses of *I. scapularis* nymphs to 0.05, 0.5, and 5 mg of guanine, uric acid, xanthine, and adenine were examined in Petri dish bioassays and replicated 10 times.

Mixtures of purines consisting of guanine, xanthine, and adenine in a 25:1:1 ratio elicited responses in *A. persicus* equivalent to the natural pheromone (Dusbábek et al., 1991a). Comparisons were made of responses of *I. scapularis* nymphs to concentrations of this purine mixture ranging from 10 to 0.001 mg.

*Analysis.* Data were transformed [ $\log(x + 1)$ ] prior to statistical analysis; however, original numbers are presented in tables and figures. Data were analyzed by ANOVA and differences between means located by paired *t* test.

## RESULTS

Cast larval skins, tick-exposed filter paper, and tick exudate elicited more assembly response in nymphs than untreated controls (Table 1). Within 1 hr, there were more ticks in sectors with larval skins ( $t = -5.93$ ,  $df = 18$ ,  $P < 0.001$ ), exposed filter paper ( $t = -9.76$ ,  $df = 18$ ,  $P < 0.001$ ), and exudate ( $t = -5.69$ ,  $df = 18$ ,  $P < 0.001$ ) compared to untreated controls. Similarly by 2 and 24 hr, there were more ticks in the sectors with larval skins ( $t = -3.99$ ,  $df = 18$ ,  $P < 0.001$ ;  $t = -4.8$ ,  $df = 18$ ,  $P < 0.001$ , respectively), exposed filter paper ( $t = -11.4$ ,  $df = 18$ ,  $P < 0.001$ ;  $t = -13.5$ ,  $df = 18$ ,  $P < 0.001$ , respectively), and exudate ( $t = -6.39$ ,  $df = 18$ ,  $P < 0.001$ ;  $t = -5.3$ ,  $df = 18$ ,  $P < 0.001$ , respectively) than in the controls. There was no difference in tick response between 1 and 24 hr in Petri dishes containing larval skins ( $t = -0.48$ ,  $df = 18$ ,  $P = 0.31$ ), exposed filter paper ( $t = 0$ ,  $df = 18$ ,  $P = 0.5$ ), exudate ( $t = 1.32$ ,  $df = 18$ ,  $P = 0.1$ ), or untreated controls ( $t = 1.34$ ,  $df = 18$ ,  $P = 0.09$ ).

TABLE 1. ASSEMBLY OF *I. scapularis* NYMPHS IN PETRI DISH SECTORS<sup>a</sup>

Time (hr)	Ticks in treatment sector (zone 1) (% , mean $\pm$ SE)			
	Untreated control	Cast skins	Exposed filter paper	Exudate
1	8.0 (2.5)	36.1 (4.0) <sup>b</sup>	40.8 (2.1) <sup>b</sup>	30.0 (2.9) <sup>b</sup>
2	4.0 (1.6)	34.2 (7.3) <sup>b</sup>	38.5 (2.5) <sup>b</sup>	24.2 (2.6) <sup>b</sup>
24	4.0 (1.6)	40.0 (7.3) <sup>b</sup>	40.2 (2.1) <sup>b</sup>	24.0 (3.4) <sup>b</sup>

<sup>a</sup>Petri dishes contained cast larval skins (10), filter paper exposed to blood-fed larvae, or exudate from molting ticks or untreated controls. Each mean represents 10 replicates with 10 nymphs per replicate.

<sup>b</sup>Significantly different from the untreated control, paired *t* test,  $P < 0.001$ .

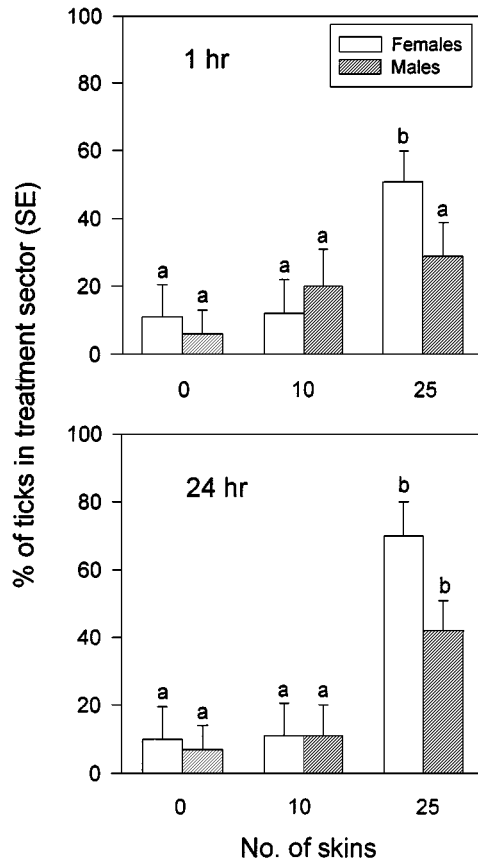


FIG. 1. Responses (mean  $\pm$  SE) of adult *I. scapularis* to numbers of cast larval skins in Petri dish bioassays. Data are averages of 10 replications. Within each sex, bars marked by the same letter are not significantly different ( $P > 0.05$ ).

Both male and female *I. scapularis* also responded to the presence of cast larval skins (Figure 1). In the presence of 10 larval skins, there was no difference after 1 hr in the number of male ( $t = -0.6$ ,  $df = 18$ ,  $P = 0.27$ ) or female ( $t = 0.24$ ,  $df = 18$ ,  $P = 0.40$ ) ticks in treatment sectors, nor was there a difference at 24 hr (male  $t = 0.0$ ,  $df = 18$ ,  $P = 0.5$ ; female  $t = 0.0$ ,  $df = 18$ ,  $P = 0.5$ ) compared to untreated controls. In the presence of 25 larval skins, there were more females in sectors with larval skins at both 1 hr ( $t = -1.93$ ,  $df = 18$ ,  $P < 0.05$ ) and 24 hr ( $t = -3.28$ ,  $df = 18$ ,  $P < 0.001$ ). There was no difference in numbers of males present in treatment sectors at 1 hr ( $t = -0.89$ ,  $df = 18$ ,  $P = 0.19$ ) compared to untreated control sectors; however, there was a difference at 24 hr ( $t = -1.4$ ,  $df = 18$ ,  $P < 0.05$ ).

TABLE 2. ASSEMBLY OF *I. scapularis* AFTER TWO-HOUR EXPOSURE TO CAST LARVAL SKINS, SALINE, OR METHANOL OR HEXANE EXTRACTS OF SKINS IN PETRI DISH BIOASSAYS<sup>a</sup>

Treatment	Ticks in treatment sector (zone 1) (% , mean $\pm$ SE)		
	Females	Males	Nymphs
Saline extract (95% NaCl)	64.3 (13.2) <sup>b</sup>	42.8 (13.7) <sup>c</sup>	45.0 ( 6.7) <sup>b</sup>
Methanol extract	10.0 (10.0)	10.0 (10.0)	10.0 (10.0)
Hexane extract	0.0 (0.0)	20.1 (13.1)	20.0 (13.3)
Cast skins	60.5 (13.3) <sup>b</sup>	40.5 (16.3) <sup>c</sup>	40.0 ( 7.3) <sup>c</sup>
Control	6.6 (6.6)	6.2 (6.2)	6.0 ( 3.1)

<sup>a</sup>Each treatment consisted of 20 skins or skin equivalents ( $N = 10$ ).

<sup>b</sup>Significantly different from the untreated control, paired  $t$  test,  $P < 0.001$ .

<sup>c</sup>Significantly different from the untreated control, paired  $t$  test,  $P < 0.05$ .

Responses of ticks were similar to cast skins and to saline extracts of the skins (Table 2). More females were present in sectors with cast skins ( $t = -3.85$ ,  $df = 18$ ,  $P < 0.001$ ) and the saline extract ( $t = -3.02$ ,  $df = 18$ ,  $P < 0.001$ ) than the untreated controls. Similarly, responses of males and nymphs were greater to the cast skins ( $t = -1.88$ ,  $df = 18$ ,  $P < 0.05$ ;  $t = -4.29$ ,  $df = 18$ ,  $P < 0.001$ , respectively) and the saline extract ( $t = -2.37$ ,  $df = 18$ ,  $P < 0.05$ ;  $t = -5.29$ ,  $df = 18$ ,  $P < 0.001$ , respectively). Methanol and hexane extracts were not any more attractive than the controls ( $P < 0.05$ ).

Assembly responses of nymphs were elicited by several of the purine standards tested (Table 3). More nymphs were present in sectors with guanine, uric acid, hypoxanthine, xanthine, inosine, and hematin compared to the solvent controls ( $P < 0.05$ ). There was no significant response to adenine compared to the controls (Table 3). In general, responses increased slightly from 1 to 24 hr. After 24 hr, responses of nymphs to cast skins ( $40.0 \pm 7.3\%$ ) were similar to responses to hematin ( $t = 1.56$ ,  $df = 18$ ,  $P = 0.07$ ), xanthine ( $t = 0.41$ ,  $df = 18$ ,  $P = 0.07$ ) and inosine ( $t = 1.51$ ,  $df = 18$ ,  $P = 0.07$ ).

Similarly, responses of adults were also elicited by purine standards (Table 4). Uric acid, hypoxanthine, adenine, and hematin were also tested, but none of these elicited responses greater than the solvent controls ( $P > 0.05$ ), and these data are not presented. More males and females were present in sectors with guanine, inosine, and xanthine compared to solvent controls (Table 4). Responses of females were similar to those obtained with cast skins (Table 2) and guanine ( $t = 0.86$ ,  $df = 18$ ,  $P = 0.19$ ) and xanthine ( $t = 0.86$ ,  $df = 18$ ,  $P = 0.18$ ); responses of males were similar between cast skins (Table 2) and guanine ( $t = -0.86$ ,  $df = 18$ ,  $P = 0.19$ ), inosine ( $t = 0.0$ ,  $df = 18$ ,  $P = 0.5$ ) and xanthine ( $t = 0.0$ ,  $df = 18$ ,  $P = 0.5$ ).

TABLE 3. ASSEMBLY OF *I. scapularis* NYMPHS IN PETRI DISH BIOASSAYS IN RESPONSE TO PURINE COMPOUNDS (0.5 mg)<sup>a</sup>

Time (hr)	Ticks in the treatment sector (zone 1) (% , mean ± SE)						
	Control	Guanine	Uric acid	Hypoxanthine	Xanthine	Inosine	Hematin
1	10.0 (4.4)	16.0 (5.0)	20.0 (5.1)	26.0 (5.1) <sup>c</sup>	22.0 (2.0) <sup>c</sup>	26.0 (4.0) <sup>c</sup>	24.0 (4.0) <sup>b</sup>
2	6.0 (3.0)	20.0 (4.8) <sup>b</sup>	30.0 (8.5) <sup>c</sup>	24.0 (5.6) <sup>b</sup>	28.0 (4.8) <sup>c</sup>	26.0 (2.4) <sup>c</sup>	24.0 (6.7) <sup>c</sup>
24	12.0 (4.4)	20.0 (5.0)	24.0 (6.0)	18.0 (6.6)	32.0 (3.7) <sup>c</sup>	36.0 (6.1) <sup>c</sup>	26.0 (5.6) <sup>b</sup>

<sup>a</sup>Each mean represents 10 replicates with 10 nymphs per replicate.

<sup>b</sup>Significantly different from the solvent control, paired *t* test, *P* < 0.05.

<sup>c</sup>Significantly different from the solvent control, *P* < 0.001.

TABLE 4. ASSEMBLY OF *I. scapularis* ADULTS TO PURINES IN PETRI DISH BIOASSAYS<sup>a</sup>

Time (hr)	Ticks in treatment sector (zone 1) (% , mean $\pm$ SE)							
	Females				Males			
	Control	Guanine	Inosine	Xanthine	Control	Guanine	Inosine	Xanthine
1	10.0 (10.0)	33.3 (12.6)	40.0 (13.1) <sup>b</sup>	33.3 (12.6)	6.0 (6.0)	33.3 (12.6) <sup>b</sup>	40.0 (13.1) <sup>b</sup>	26.6 (11.8)
2	6.0 (6.0)	33.3 (12.6) <sup>b</sup>	26.6 (11.8)	33.3 (12.6) <sup>b</sup>	6.0 (6.0)	40.0 (12.6) <sup>b</sup>	46.6 (13.3) <sup>b</sup>	33.3 (12.6) <sup>b</sup>
24	10.0 (10.0)	40.0 (16.4) <sup>b</sup>	20.0 (13.3)	40.0 (16.4) <sup>b</sup>	6.0 (6.0)	60.0 (16.4) <sup>c</sup>	40.0 (16.4) <sup>b</sup>	40.0 (16.0) <sup>b</sup>

<sup>a</sup>Each mean represents 15 replicates.

<sup>b</sup>Significantly different from the solvent control, paired *t* test, *P* < 0.05.

<sup>c</sup>Significantly different from the solvent control, paired *t* test, *P* < 0.001.

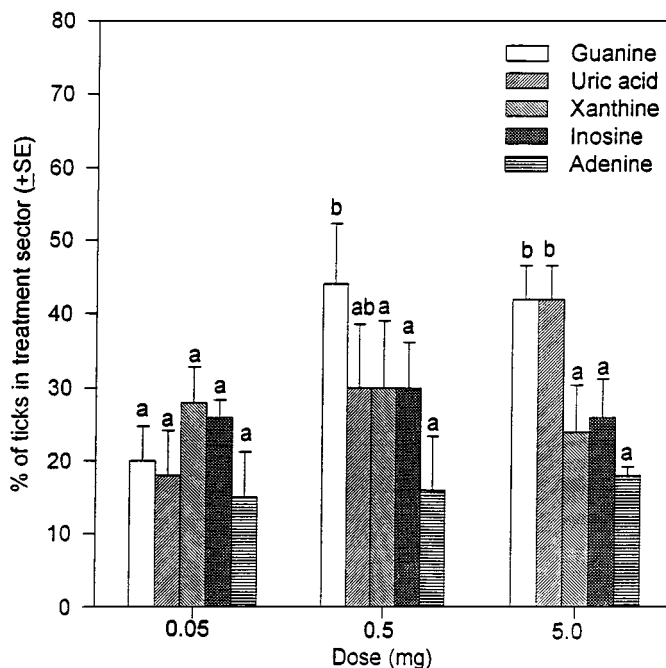


FIG. 2. Responses (mean  $\pm$  SE) of *I. scapularis* nymphs to different concentrations of purines in a Petri dish bioassay. Data are averages of 10 means. Within each compound, bars marked by the same letter are not significantly different ( $P > 0.05$ ).

Responses to several purine standards increased with dose when tested against nymphs (Figure 2). Differences in responses with increased dose were observed with guanine ( $F = 5.51$ ,  $df = 2, 29$ ,  $P < 0.001$ ) and uric acid ( $F = 3.43$ ,  $df = 2, 29$ ,  $P = 0.04$ ), but not with adenine ( $F = 0.03$ ,  $df = 2, 29$ ,  $P = 0.96$ ), xanthine ( $F = 0.20$ ,  $df = 2, 29$ ,  $P = 0.81$ ), or inosine ( $F = 0.23$ ,  $df = 2, 29$ ,  $P = 0.29$ ).

Responses of nymphs to a range of concentrations of a mixture of guanine, xanthine, and adenine (25:1:1 ratio) differed (Figure 3) ( $F = 2.55$ ,  $df = 4, 74$ ,  $P = 0.04$ ). Mixtures consisting of purines at 10.0, 1.0, and 0.1 mg elicited the highest responses (Figure 3). Responses of nymphs to cast skins were not different than responses to 10 mg and 1 mg ( $t$  test,  $P > 0.05$ ) of purine mixtures, but were greater than other the other concentrations of purine mixtures tested ( $t$  test,  $P < 0.05$ ).

#### DISCUSSION

This study provides clear evidence of an assembly pheromone in *Ixodes scapularis*. Similar to other reported assembly pheromones in ticks (summarized

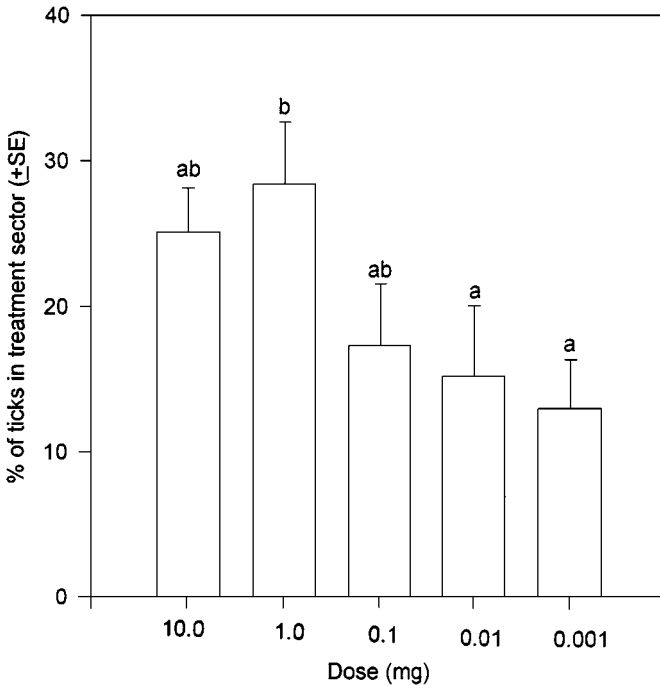


FIG. 3. Responses (mean  $\pm$  SE) of *I. scapularis* nymphs to different concentrations of a mixture of guanine, xanthine, and adenine in a 25:1:1 ratio. Data are averages of 10 replicates in Petri dish bioassays. Bars marked by the same letter are not significantly different ( $P > 0.05$ ).

in Sonenshine, 1991), arrestment responses were elicited by tick waste products (excreta and cast skins), responses could be elicited by saline but not organic solvent extracts, and responses were not stage-specific, with significant responses elicited from males, females, and nymphs. Additionally, equivalent assembly responses could be elicited in both nymphs and adults to several purine compounds previously identified as components of assembly pheromones in soft ticks (Dusbábek et al., 1991a).

Excretion of nitrogenous waste products by ticks generally occurs after blood-feeding and immediately after molting (thus contaminating the cast skins). Locations with an accumulation of excretory products are likely conducive to tick survival as these indicate successful completion of blood feeding and molting and have appropriate environmental conditions for tick survival (Otieno et al., 1985). Although there may be differences in sites of molting of different stages of *I. scapularis*, arrestment of tick movement in sites containing excretory products may result in assemblies of ticks of different stages since assembly pheromones are

nonspecific in activity. Although activity is nonspecific, there may be differences in quantities and mixtures of constituents produced between different stages. This study was conducted using cast skins from blood-fed larvae because of their use in previous studies and the ability to produce large numbers of them.

Nitrogenous waste products from ticks contain primarily guanine, other purines in lower concentrations and hematin (Hamdy, 1972, 1973). Dusbábek et al. (1991a) reported the relative abundance of guanine, xanthine, and hypoxanthine in excretory granules of five soft tick species. Several studies associated the observed assembly behaviors with excretory wastes of ticks (Leahy et al., 1973, 1975; George, 1981; Hájková and Leahy, 1982; Otieno et al., 1985; Dobrotvorskii et al., 1991) as well as mites (Entrekin and Oliver, 1982; Arlian and Vyszenski-Moher, 1996). Aggregation in response to guanine was demonstrated in *Argas persicus* (Otieno et al., 1985), *Argas walkerae* (Neitz and Gothe, 1984), and *Argas persicus* (Hassanali et al., 1989). Response to guanine alone was reported by Gothe (1987) to be lower than to naturally occurring pheromone, supporting the idea that additional components may be present. Other compounds such as xanthine, hypoxanthine, and uric acid also elicited significant responses with response levels high in Otieno et al. (1985) (>75%). In addition to guanine, Dusbábek et al. (1991a) also reported significant assembly responses (>55%) of *A. persicus* males to xanthine, hypoxanthine, and inosine. Compounds tested that did not elicit assembly of ticks included adenine (Otieno et al., 1985; Dusbábek et al., 1991a), adenosine, guanosine, and uric acid (Dusbábek et al., 1991a). Several of these compounds, however, were reported to elicit assembly responses in *Sarcoptes* mites (adenine, uric acid) (Arlian and Vyszenski-Moher, 1996). Our data provide clear evidence that guanine, uric acid, hypoxanthine, xanthine, inosine, and hematin elicit assembly responses in *I. scapularis* in immatures and adults. However, whether any of these compounds occur in *I. scapularis* excreta and if so, their relative frequency, has yet to be determined.

Mixtures of purines appeared to elicit more assembly in the soft tick, *A. persicus*, compared to the purines alone (Dusbábek et al., 1991a). A mixture of guanine, adenine, and xanthine mixed in a ratio of 25:1:1 enhanced assembly of ticks equivalent to about 75% of the natural pheromone. Our data indicate that assembly of *I. scapularis* to a similar mixture of purines appears to be dose-dependent, and the highest responses to mixtures are equivalent to those of cast skins. The future identification of components and ratios in extracts may aid in further enhancement of assembly response in purine mixtures for *I. scapularis*.

*Ixodes scapularis* has emerged as a common suburban pest in the northeastern United States with the increased encroachment of communities into wooded areas (Lastavica et al., 1989; Carroll et al., 1992). Human risk for Lyme disease depends on the density of infected ticks as well as the degree of human-tick contact (Mather et al., 1989) with most risk associated with exposure to nymphs in summer (Spielman et al., 1985). Most tick control strategies are areawide and

do not specifically target ticks. The identification of assembly pheromones in *I. scapularis* can provide the basis for development of an alternative targeted control with the potential to reduce overall acaricide use and nontarget effects. The potential for such an approach was demonstrated by initial studies by Dusbábek et al. (1997), who reported the enhancement of efficacy of permethrin against immatures and adults of *A. persicus* when used in conjunction with guanine hydrochloride.

Future research is needed to identify components in saline extracts containing the assembly pheromone and to determine a mixture of components equivalent in response to natural pheromone. Formulation of such components, in conjunction with an acaricide and perhaps also with volatile attractant compounds, may serve as the basis for development of a targeted control approach for *I. scapularis*.

*Acknowledgments*—The senior author gratefully acknowledges the American Lyme Disease Foundation and the College of Veterinary Medicine, University of Florida, for providing funding for this research. This is the Florida Agricultural Experiment Station Journal Series no. R-08337.

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